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Abstract Drug-induced inhibition of mitochondrial fatty acid β-oxidation (mtFAO) is a key mechanism whereby drugs can induce steatosis. The type and severity of this liver lesion is dependent on the residual mtFAO flux. Indeed, a severe inhibition of mtFAO is leading to microvesicular steatosis, hypoglycemia and liver failure, which can be favored by genetic predispositions. In contrast, moderate impairment of mtFAO can cause macrovacuolar steatosis, which is by itself a benign lesion. In the long-term, however, macrovacuolar steatosis can progress with some drugs to steatohepatitis. Interestingly, drugs that are more likely to cause steatohepatitis are those impairing the mitochondrial respiratory chain (MRC) activity. Indeed, MRC impairment favors not only hepatic fat accretion but also oxidative stress and lipid peroxidation. Drugs inhibiting mtFAO could be more toxic in obese patients with preexisting nonalcoholic fatty liver disease (NAFLD) since higher mtFAO is a key metabolic adaptation to curb fat accretion during NAFLD.

Introduction

Drug-induced liver injury (DILI) includes several kinds of lesions such as acute hepatitis, cholestasis, phospholipidosis and steatosis [1•, 2]. This latter lesion corresponds to accretion of lipids, mainly triglycerides, although other lipids species can also accumulate such as free fatty acids (FAs) and acyl-carnitine derivatives [3, 4]. Actually, drugs are able to induce either microvesicular steatosis or macrovacuolar steatosis, and sometimes both kinds of lipid deposition [3, 5]. In this review, we will discuss how drug can induce hepatic steatosis by disturbing the mitochondrial fatty acid oxidation (mtFAO) pathway [3-7]. However, before considering drug-induced hepatic steatosis, we will recall key features of mtFAO and its regulation.

mtFAO and Oxidative Phosphorylation

mtFAO and oxidative phosphorylation (OXPHOS) have been reviewed previously in details [3, 8, 9]. Briefly, mitochondria provide most of ATP by way of the oxidation of substrates such as FAs and pyruvate. Whereas pyruvate oxidation takes place in the tricarboxylic acid (TCA) cycle, mitochondrial degradation of FAs is mediated by the β-oxidation pathway. To this end, FAs must cross the mitochondrial membranes. Whereas short-chain and medium-chain fatty acids (SCFAs/MCFAs) freely enter the mitochondria and are then activated into acyl-CoA molecules by specific acyl-CoA synthetases, long-chain fatty acids (LCFAs) must cross the mitochondrial membranes with a multi-enzymatic system requiring both coenzyme A (CoA) and L-carnitine as cofactors. In this system, carnitine palmitoyltransferase 1 (CPT1) and CPT2 are playing a major role (Fig. 1). Inside mitochondria, acyl-CoA derivatives are cut down sequentially by the β-oxidation process that generates acetyl-CoA moieties and shorter fatty acids that enter new β-oxidation cycles (Fig. 1). These acetyl-CoA moieties subsequently generate ketone bodies (KB), which are used for ATP synthesis in extra-hepatic tissues. The key role of mtFAO in energy homeostasis is illustrated by the occurrence of multiple organ failure and death when this metabolic pathway is severely affected [3, 4, 10].

mtFAO produces not only acetyl-CoA molecules but also NADH and $FADH_2$ that provide their electrons and protons to the mitochondrial respiratory chain (MRC) (Fig. 1). This transfer of electrons and protons allows the regeneration of NAD^+ and FAD, and the synthesis of ATP from ADP (Fig. 1). The whole process coupling substrate oxidation to ATP synthesis is called OXPHOS. OXPHOS uncouplers are drugs (or chemicals) that can reduce the mitochondrial membrane potential ($\Delta\psi_m$) and abolish ATP synthesis without inhibiting substrate oxidation [3, 4, 11, 12•, 13].

A unique feature of mitochondria is that 13 MRC polypeptides are encoded by mitochondrial DNA (mtDNA) (Fig. 1). There are several hundred copies of mtDNA in a single cell and mtDNA replication is carried out by the DNA polymerase γ [3, 5, 8]. In liver, it is deemed that mtDNA copy number must fall below 20 to 40% of basal level in order to induce MRC impairment [14, 15], which can secondarily lead to reactive oxygen species (ROS) overproduction [16, 17]. Conversely, ROS and other endogenous molecules such as reactive nitrogen species (RNS) can subsequently damage mtDNA, thus leading to mtDNA mutations and depletion [5, 15, 18].

Regulation of the mtFAO Pathway

During fasting, the expression of many enzymes involved in mtFAO is up-regulated by peroxisome proliferator-activated receptor α (PPARα), a transcription factor which can be activated by endogenous FAs [5, 9]. In addition, other transcription factors positively regulating hepatic FAO during fasting include forkhead box A2 (FoxA2) and cAMP-response element-binding protein (CREB) [5, 19]. Moreover, the PPARγ coactivators-1α and 1β (PGC-1α/β) are playing a key role in the transcriptional regulation of mtFAO enzymes [5, 9, 19]. After a meal, mtFAO of LCFAs can be inhibited by malonyl-CoA, since this intermediate of lipogenesis strongly inhibits CPT1 [3, 9].

In pathophysiological conditions, other negative regulations can exist. For instance, any significant reduction in CoA and L-carnitine levels can compromise mtFAO [3, 4, 20]. A strong reduction of MRC activity can also impair mtFAO. Indeed, inhibition of MRC activity slows down the oxidation of NADH and $FADH_2$ into NAD^+ and FAD, which are mandatory cofactors for several mtFAO dehydrogenases [3, 21, 22]. Furthermore, any significant reduction of MRC activity can also impair the TCA cycle and cause lactic acidosis [4, 23].

Drug-Induced Inhibition of mtFAO

The main mechanisms whereby drugs are able to inhibit mtFAO can be classified into five different categories. It is noteworthy that different mechanisms can be involved for the same drug.

Direct Inhibition of Mitochondrial β-Oxidation Enzyme(s)

Some drugs (or their metabolites) can directly inhibit one or several enzyme(s) involved in mtFAO (Fig. 1). This has been showed with amiodarone, tamoxifen, perhexiline and valproic acid (VPA), or suspected with ibuprofen, amineptine and tianeptine [3-5, 24-28, 29•]. For the latter three drugs, a

stronger inhibition of mitochondrial β-oxidation of SCFAs and MCFAs compared to LCFAs suggested a specific impairment of enzymes involved in SCFA and MCFA oxidation, although the investigations did not determine the exact targeted enzyme(s) [24, 25].

Regarding VPA (dipropylacetic acid), severe inhibition of mtFAO is probably due to the generation of Δ2,4-VPA-CoA and other reactive metabolites that irreversibly inactivate FAO enzyme(s) [3, 30]. Interestingly, acetaminophen (APAP) could inhibit mtFAO and MRC activity *via* the generation of N-acetyl-*p*-benzoquinone imine (NAPQI) [31, 32], a reactive metabolite generated by cytochromes P450 3A4 and 2E1 (CYP2E1) [33]. This may explain, with other mechanisms (see below), why APAP intoxication can induce steatosis in rodents [34-36] and in some individuals (Table 1) [1•, 2].

Some investigations allowed the identification of the mtFAO enzyme(s) inhibited by the aforementioned drugs. For instance, it has been shown that CPT1 can be inhibited by VPA, amiodarone and tamoxifen [28, 29•, 37]. Troglitazone is able to inhibit long-chain acyl-CoA synthase (ACS) (Fig. 1), thus impairing the mitochondrial entry of LCFAs through a CPT1-independent mechanism [38].

Sequestration of CoA and/or L-Carnitine

Drugs such as VPA, salicylic acid and ibuprofen can impair mtFAO *via* the generation of CoA and/or L-carnitine esters, which decreases the availability of these cofactors for the β-oxidation of endogenous FAs (Fig. 1) [3, 5, 25, 39]. However, drug-induced inhibition of mtFAO secondary to CoA and/or L-carnitine depletion could occur only when cellular levels of these cofactors are already below physiological concentrations [25, 39]. For some drugs, generation of xenobiotic acyl-CoA thioesters could also competitively inhibit different mitochondrial enzyme(s) [40].

Inhibition of the MRC

mtFAO can also be secondarily impaired as a result of severe inhibition of the MRC [3-5]. This could occur with amiodarone, perhexiline, tamoxifen and buprenorphine [4, 21, 26, 28, 41]. Interestingly, these amphiphilic drugs can be protonated within the mitochondrial intermembrane space, thus generating cationic compounds entering the matrix thanks to $\Delta\psi_m$ (Fig. 1). This allows their accumulation within mitochondria and the subsequent inhibition of mtFAO and MRC enzymes. Actually, whereas relatively low concentrations of these amphiphilic drugs can inhibit directly FAO enzyme(s), higher concentrations are required to impair the respiratory chain [11, 21, 26, 28, 41]. Thus, mitochondrial accumulation of these amphiphilic drugs eventually inhibits FAO through a dual

mechanism. The precise sites of MRC inhibition have been identified for amiodarone and perhexiline, which both inhibit complexes I and II [21, 26].

For some drugs inhibiting mtFAO, investigations have not been able to clearly establish whether this deleterious effect was due to direct inhibition of mtFAO enzyme(s), or to indirect inhibition *via* MRC impairment. This is the case for tetracycline derivatives for which *bone fine* inhibition of mtFAO has been shown in some investigations [42, 43], while MRC (or OXPHOS) impairment has been demonstrated in other independent studies [3, 44, 45].

Drugs such as tianeptine and ibuprofen inhibit MRC activity, in particular at the level of complex I [46-48]. Importantly, these effects were demonstrated on heart, duodenum or brain mitochondria. However, some investigations showed that mitochondrial toxicity could greatly vary between tissues [15, 49]. Actually, ibuprofen and tianeptine-induced impairment of complex I in liver mitochondria is unlikely to be strong because this would cause a similar inhibition of β-oxidation with all kinds of FAs (i.e. SCFAs, MCFAs and LCFAs), thus irrespective of their chain length. However, investigations performed on isolated mouse liver mitochondria showed that these drugs inhibited more strongly the β-oxidation of SCFAs and MCFAs compared to LCFAs [24, 25]. Accordingly, although ibuprofen inhibited mitochondrial respiration on isolated liver mitochondria (Table 2) [12•], this effect appeared with much higher concentrations compared to those affecting MRC activity with mitochondria isolated from duodenum [48].

Impairment of mtDNA Replication

Inhibition of mtFAO can also result from reduced hepatic mtDNA levels. This mechanism has been shown for the antiviral fialuridine (FIAU), zidovudine (AZT), stavudine (d4T) and didanosine (ddI), which all inhibit mtDNA polymerase γ activity [3-5, 50, 51]. Importantly, liver injury induced by these drugs can be associated with severe lactic acidosis, which is caused by TCA cycle inhibition. Interestingly, tamoxifen and tacrine can induce hepatic mtDNA depletion possibly by interacting with the mitochondrial topoisomerases [28, 52].

Finally, some drugs could also induce mtDNA depletion *via* the generation of ROS, RNS and/or reactive metabolites. For instance, APAP and troglitazone can reduce mtDNA levels by inducing mtDNA strand breaks [53, 54]. Indeed, damaged mtDNA molecules harboring numerous strand breaks (or other bulky damages) can be rapidly degraded by mitochondrial endonucleases [18, 55].

Impaired PPARα Activity

Some drugs could impair mtFAO by reducing PPARα expression and activity. This is suspected with APAP, VPA and tetracycline that reduce the mRNA expression of PPARα and some of its target genes including CPT1 [36, 44, 56]. Although the direct consequences of this effect are still uncertain, impairing PPARα activity could prevent an important metabolic adaptation that takes place during drug-induced steatosis [57, 58]. The mechanism(s) whereby these drugs could impair PPARα expression might deserve further attention.

Drug-Induced Alterations of Other Pathways Involved in Lipid Homeostasis

Although not being in the scope of this article, it is noteworthy that drug-induced steatosis can be also caused by other mechanisms. For instance, drugs can inhibit very low density lipoprotein (VLDL) secretion and increase lipid synthesis, in particular by direct (or indirect) activation of key lipogenic transcription factors such as sterol regulatory element binding protein 1c (SREBP-1c) and PPARγ [5, 59, 60]. Interestingly, some drugs such as amiodarone and tamoxifen could both inhibit mtFAO and stimulate *de novo* lipogenesis [5, 61•].

Drug-Induced Microvesicular Steatosis

Numerous investigations have shown that drug-induced microvesicular steatosis is the consequence of severe inhibition of mtFAO [3-7, 21, 24, 25, 28, 29•, 39, 42, 62]. Interestingly, this mechanism is also involved in the pathophysiology of microvesicular steatosis occurring in other conditions such as inborn errors of mtFAO, Reye's syndrome and acute fatty liver of pregnancy [3, 10].

A primary consequence of severe inhibition of mtFAO is ATP depletion and accumulation of FAs that are either esterified into triglycerides, or that remain as a free form [3, 13]. It has been postulated that the small size of the lipid droplets could be due to an "emulsification" of triglycerides by free fatty acids [3], although this hypothesis has never been confirmed. Alternatively, the nature and/or the abundance of some proteins wrapping the lipids could play a role [5, 63]. Whereas triglycerides are not toxic for the cells, free FAs and some of their derivatives (i.e. acyl-CoA thioesters and dicarboxylic acids) could worsen mitochondrial dysfunction and cause cell death [3, 9, 64].

Drug-induced microvesicular steatosis is a potentially severe and fatal liver lesion that can be associated with liver failure, encephalopathy and profound hypoglycemia [3-5]. Liver pathology shows the presence within the cytoplasm of numerous lipid droplets, which leave the nucleus in the center of the hepatocyte [3, 21, 42, 62]. Besides lipid accumulation, hepatic cytolysis and increased plasma

transaminases can also be observed to a variable degree. Examples of drugs able to induce microvesicular steatosis are given in Table 1 [3, 4, 5, 65-68].

Drug-induced microvesicular steatosis can be associated with severe hypoglycemia and abnormal levels of plasma KB [3-5]. Hypoglycemia could be due to impaired gluconeogenesis and/or to higher extra-hepatic utilization of glucose [3, 69]. Although hypoketonemia has been observed with VPA, pirprofen and ibuprofen, high levels of plasma KB was also reported experimentally with amineptine, amiodarone, salicylic acid, tetracycline and tianeptine [3, 4, 21, 24, 39, 42, 70]. Drug-induced hyperketonemia could be related to a severe inhibition of peripheral KB utilization [3, 4]. Finally, microvesicular steatosis can be associated with an accumulation of acyl-carnitine derivatives and dicarboxylic acids in plasma and urine [3-5].

Inhibition of mtFAO and Steatosis Induced by Other Xenobiotics

Although beyond the scope of this review, it is noteworthy that non-pharmaceutical compounds are able to inhibit mtFAO and induce hepatic lipid accumulation, especially as microvesicular steatosis. It is for instance the case with alcohol, cocaine, perfluorooctane sulfonate (a persistent organic pollutant), triptolide (a diterpenoid epoxide isolated from a Chinese woody wine plant), and hypoglycine (a toxin present in the unripe fruit of Jamaican ackee tree) [3, 55, 71•, 72, 73].

Factors Favoring Drug-Induced Mitochondrial Dysfunction

At least three factors could favor drug-induced impairment of mtFAO and MRC activity. Importantly, these factors are not mutually exclusive and their combination is likely to induce severe mitochondrial dysfunction and microvesicular steatosis in some patients.

1) *Drug structure and biotransformation*. Amiodarone, perhexiline and tamoxifen are amphiphilic drugs harboring protonable amine moieties that favor their accumulation inside the mitochondrial matrix [3, 7, 11, 26, 28]. For amiodarone, the benzofuranyl-phenylmethanone moiety could be the chemical structure responsible for mitochondrial dysfunction [74, 75, 76•]. VPA is a branched-chain fatty acid that freely enters the mitochondria, where it is activated by CoA and undergoes β-oxidation [3, 6, 40]. However, the two-step biotransformation of VPA by CYPs and β-oxidation generates reactive metabolites that irreversibly inactivate FAO enzymes and induce cytotoxicity [3, 30, 77]. Regarding the role of CYPs, it is noteworthy that APAP-induced dysfunction of liver mitochondria could depend, at least in part, on the presence of CYP2E1 within these organelles [32]. Finally, the antiretroviral nucleoside reverse transcriptase inhibitors (NRTIs) are able to inhibit mtDNA replication and cause severe mtDNA depletion owing to their structural analogy with the natural nucleosides [3,

6]. In this pharmacological class, drugs such as zalcitabine (ddC), ddI and d4T are significantly more toxic to mitochondria than others (AZT and lamivudine) [78].

2) *Drug dosage and duration of treatment*. Clinical reports in the 50's and 60's indicated that severe microvesicular steatosis induced by tetracycline and its derivatives was clearly dose-dependent [3]. In particular, most cases of steatosis were observed in patients receiving large intravenous dosages (>1.5 g/day) of tetracycline derivatives [3]. However, tetracycline-induced steatosis is no longer observed since such huge intravenous doses have been abandoned. Long-lasting administration of NRTIs also increases the risk of mitochondrial toxicity in liver and other tissues [15, 79].

3) *Genetic predispositions*. Several congenital defects in mtFAO and OXPHOS enzymes have been detected in patients with VPA-induced hepatotoxicity [3, 5, 80]. A mutation in the gene encoding DNA polymerase γ (POLG) could favor mitochondrial toxicity induced by NRTIs, possibly by enhancing the probability of their incorporation within the mtDNA molecules and the subsequent arrest of mtDNA replication [5, 81]. Intriguingly, mutations in the POLG gene could also favor VPA-induced hepatotoxicity [82•], although this cannot be explained by the incorporation of VPA within mtDNA. Finally, interindividual differences in mitochondrial antioxidant enzymes such as MnSOD could enhance the risk of mitochondrial toxicity and liver injury [83, 84].

Drug-Induced Macrovacuolar Steatosis and Steatohepatitis

With some drugs, liver triglycerides accumulate as a large (often single) lipid vacuole displacing the nucleus close to the plasma membrane. This lesion is commonly referred to as macrovacuolar steatosis and can be induced by other factors such as high-calorie feeding and ethanol intoxication [4, 83, 85]. Examples of drugs able to induce macrovacuolar steatosis are indicated in Table 1 [4, 5, 86-88].

The prevalence of drug-induced macrovacuolar steatosis may be underestimated, mainly because this liver lesion is benign, at least in the short term. However, this lesion can progress in the long term to steatohepatitis, which is characterized by necrosis, inflammation and some fibrosis. Moreover, some drugs such as amiodarone, perhexiline, tamoxifen and didanosine (ddI) can also induce cirrhosis after long-term treatment (Table 1) [1•, 5].

In contrast to microvesicular steatosis that can be considered as a *bona fide* mitochondrial disease, several mechanisms seem to be involved in the pathogenesis of drug-induced macrovacuolar steatosis. These mechanisms include moderate inhibition of mtFAO, enhanced *de novo* lipogenesis and reduced secretion of VLDL [5, 59, 60]. Importantly, these different mechanisms are not mutually exclusive, and for instance some drugs can inhibit both mtFAO and VLDL secretion [5, 60].

It is also noteworthy that some drugs can induce both microvesicular and macrovacuolar steatosis (Table 1). Although the exact reason of this observation is unclear, it is conceivable that

microvesicular steatosis could occur when mtFAO is severely inhibited, whereas macrovacuolar steatosis could take place if mitochondrial function is relatively preserved [5]. The differences in the severity of drug-induced mitochondrial dysfunction can be explained by several factors, as discussed in the previous section.

Although the pathophysiology of drug-induced steatohepatitis is not fully understood, some data suggest that reduced MRC activity could be involved [4, 5, 26, 89]. Indeed, MRC inhibition could not only contribute to fat deposition but also to ATP deficiency, which can cause necrosis. Moreover, MRC impairment can be associated with ROS overproduction, a key event involved in the progression of fatty liver to steatohepatitis whatever its etiology [5, 9, 89]. Importantly, ROS overproduction in a cellular environment enriched in fat can trigger lipid peroxidation and the production of reactive aldehydes that induce many deleterious effects in liver [5, 9, 89, 90]. Although drug-induced inhibition of MRC and β-oxidation mostly leads to the hepatic accretion of saturated fatty acids, some polyunsaturated fatty acids can also accumulate in sufficient quantity to generate lipid peroxidation-derived reactive aldehydes in the presence of ROS [71].

Drug-Induced Inhibition of mtFAO and Aggravation of NAFLD

Obesity is often associated with metabolic disorders such as nonalcoholic fatty liver disease (NAFLD), dyslipidemia and type 2 diabetes [5, 9]. During NAFLD, insulin resistance and adaptive hyperinsulinemia favor fat deposition in liver, in particular *via* SREBP1c-mediated *de novo* lipogenesis [9]. However, there is a compensatory stimulation of mtFAO in order to limit fat accretion [9, 85, 91]. Thus, any significant impairment of mtFAO is likely to aggravate NAFLD in obese individuals. Moreover, drugs that alter MRC activity are also likely to promote the progression of fatty liver to nonalcoholic steatohepatitis (NASH) by enhancing ROS generation and oxidative stress [59].

Drugs that are suspected to aggravate NAFLD in obese patients are tamoxifen, raloxifene, irinotecan, methotrexate and NRTIs such as stavudine and didanosine [9, 59, 92]. Interestingly, inhibition of mtFAO has been documented with several of these drugs, namely tamoxifen, raloxifene and NRTIs [3, 28, 93, 94]. Moreover, drugs such as tamoxifen, methotrexate and NRTIs can inhibit MRC activity and favor oxidative stress [3, 16, 28, 95, 96]. However, for some of these compounds, aggravation of preexisting NAFLD could also be secondary to other mechanisms. For instance, tamoxifen could also inhibit VLDL secretion and stimulate *de novo* lipogenesis [28, 59, 97].

We took advantage of the present article to determine whether β-oxidation of palmitoyl-L-carnitine could be more severely inhibited by some of the afore-mentioned drugs when liver mitochondria were isolated from ob/ob mice compared to wild-type mice. Although irinotecan, methotrexate and tamoxifen impaired palmitoyl-L-carnitine β-oxidation, this inhibition was not

statistically different between wild-type and ob/ob liver mitochondria (Table 2). Nevertheless, further investigations will be required to determine whether chronic administration of these drugs could aggravate fatty liver in ob/ob mice. In contrast, other drugs were able to inhibit palmitoyl-L-carnitine β-oxidation more strongly on ob/ob liver mitochondria, as discussed below.

Conclusion and Outlook

As discussed in this review, inhibition of mtFAO is a key mechanism whereby drugs can induce steatosis and actually drug-induced microvesicular steatosis can be considered as a mitochondrial disease [3-7]. Moreover, long-term impairment of MRC activity could be an important mechanism leading to drug-induced steatohepatitis, in particular as a result of mitochondrial ROS overproduction [5, 26, 89]. It is also noteworthy that some drugs able to induce mitochondrial dysfunction could be more toxic in obese patients with preexisting NAFLD, which could be aggravated during the treatment. Indeed, drug-induced mtFAO impairment can impede a key compensatory metabolic pathway set up during NAFLD in order to limit hepatic fat accumulation [9, 59, 91], whereas MRC impairment is able to major oxidative stress and lipid peroxidation [26, 89].

Although numerous drugs can induce steatosis [1, 3, 4], their ability to inhibit mtFAO is still unknown for a majority of them. Thus, high-throughput screening can be suited in order to determine whether inhibition of mtFAO is a frequent feature observed with all the steatogenic drugs. Importantly, such screening could also assess the ability of these drugs to impair MRC activity [12•, 98•]. These investigations can also be performed on liver mitochondria isolated from obese and wild-type mice in order to determine whether “obese” mitochondria are more sensitive to drug-induced mitochondrial dysfunction. Table 2 gives some examples of steatogenic drugs and their ability to inhibit (or not) mitochondrial respiration assessed with glutamate/malate and palmitoyl-L-carnitine/malate on liver mitochondria isolated from lean and ob/ob mice.

These investigations provided some new interesting findings. For instance, diclofenac and ibuprofen-induced inhibition of mtFAO was stronger with ob/ob mitochondria (Table 2). Importantly, this stronger impairment was not due to lower basal oxygen consumption with palmitoyl-L-carnitine since it was increased by 62% in ob/ob mitochondria as compared to wild-type mitochondria, in keeping with previous investigations [9, 91]. Moreover, diclofenac induced a stronger inhibition of glutamate/malate-driven respiration on ob/ob mitochondria. It would be interesting to determine whether these two NSAIDs could worsen fatty liver in ob/ob mice, or in other murine models of obesity and NAFLD. Our investigations also revealed that irinotecan strongly inhibited mtFAO and MRC activity (Table 2). Although more investigations will be needed, these novel data could explain why this antineoplastic drug is able to induce steatohepatitis in some patients (Table 1) [2, 99]. Finally,

we found that AZT inhibited palmitoyl-L-carnitine-driven respiration at concentrations below those required to impair MRC (Table 1). Interestingly, some investigations already showed that this antiretroviral drug was able to directly impair MRC activity (e.g. complex II), in addition to its long-term deleterious effect on mtDNA replication [15]. Thus, AZT could also directly inhibit mtFAO in liver, in addition to its detrimental effects on MRC activity.

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Compliance with Ethics Guidelines

Conflict of Interest

Julie Massart, Karima Begriche and Bernard Fromenty have no conflict of interest to declare regarding this article.

Nelly Buron, Mathieu Porceddu and Annie Borgne-Sanchez are co-founders of Mitologics SAS.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Table 1

Examples of drugs inducing microvesicular steatosis, macrovacuolar steatosis, steatohepatitis or cirrhosis in treated patients.

Drug	Therapeutic class	Liver lesions
Acetaminophen (overdose)	Analgesic, antipyretic	MiSt[1], MaSt
Amineptine	Antidepressant	MiSt
Amiodarone	Antianginal, antiarrhythmic	MiSt, MaSt, StH, Cir
Aspirin (and salicylic acid)	NSAID	MiSt
Carbamazepine	Antiepileptic	MaSt, StH, Cir
Diclofenac	NSAID	MaS, Cir
Didanosine (ddI)	Antiretroviral (anti-HIV)	MiSt, MaSt, StH, Cir
Enalapril	Antihypertensive (ACE inhibitor)	MiSt
Fialuridine (FIAU)	Antiviral (anti-HBV)	MiSt
5-Fluorouracil	Antineoplastic (colorectal cancer)	MaSt, Cir
Glucocorticoids	Anti-inflammatory	MaSt
Ibuprofen	NSAID	MiSt, MaSt, Cir
Indinavir	Antiretroviral (anti-HIV)	MiSt
Interferon-α	Antiviral (anti-HCV and anti-HBV)	MaSt
Irinotecan	Antineoplastic (colorectal cancer)	MaSt, StH
Methotrexate	Antipsoriatic, anti-rheumatoid	MaSt, StH, Cir
Nifedipine	Antianginal, antihypertensive	MaSt, StH
Panadiplon	Anxiolytic	MiSt
Perhexiline	Antianginal	MaSt, StH, Cir
Pirprofen	NSAID	MiSt
Raloxifene	SERM, anti-osteoporotic	MaSt
Stavudine (d4T)	Antiretroviral (anti-HIV)	MiSt, MaSt, StH, Cir
Tamoxifen	SERM, antineoplastic (breast cancer)	MaSt, StH, Cir
Tetracycline and its derivatives (high doses)	Antibiotics	MiSt
Tianeptine	Antidepressant	MiSt
Toremifene	SERM, antineoplastic (breast cancer)	MaSt
Troglitazone	Antidiabetic	MiSt
Valproic acid	Antiepileptic	MiSt
Zidovudine (AZT)	Antiretroviral (anti-HIV)	MiSt

[1]Abbreviations in the table: ACE: angiotensin-converting enzyme; Cir, Cirrhosis; HBV, hepatitis B virus; HCV, hepatitis V virus; HIV, human immunodeficiency virus; MaSt, macrovacuolar steatosis; MiSt, microvesicular steatosis; NSAID, nonsteroidal anti-inflammatory drug; SERM, selective estrogen receptor modulator; StH, Steatohepatitis. Drugs in italics have been shown to impair mitochondrial β-oxidation and/or other key mitochondrial functions such the MRC activity. Information concerning liver lesions can be found mainly in references [1-7] and [100].

Table 2

Drug-induced inhibition of mitochondrial respiration with glutamate/malate and palmitoyl-L-carnitine/malate in liver mitochondria isolated from lean and ob/ob mice[1].

Drugs	Lean mice		Ob/ob mice	
	Glutamate + malate	**Palmitoyl-L-carnitine + malate**	**Glutamate + malate**	**Palmitoyl-L-carnitine + malate**
Acetaminophen (APAP)	>400[2]	>400	>400	>400
Carbamazepine	>400	>400	379	341
Diclofenac	35	47	16*	11*
Ibuprofen	107	287	99	80*
Irinotecan	10	6	25	10
Methotrexate	53	44	40	42
Salicylic acid	>400	>400	>400	>400
Tamoxifen	42	4	72	11
Zidovudine (AZT)	309	79	144	83

[1]Measurement of oxygen consumption in the presence of ADP (state 3) and the different substrates was carried out on the Mitologics' screening platform, as previously described [12•]. Whereas glutamate/malate-driven mitochondrial respiration assesses the MRC activity from complex I to complex IV, palmitoyl-L-carnitine/malate-driven respiration evaluates LCFA mtFAO. [2]Numbers in this table correspond to the effective concentrations (in μM) inducing 20% of the maximal effects (EC_{20}) as described in [12•]. Values are means for 3 to 5 different mitochondrial preparations. *Different from lean mice (P<0.05).

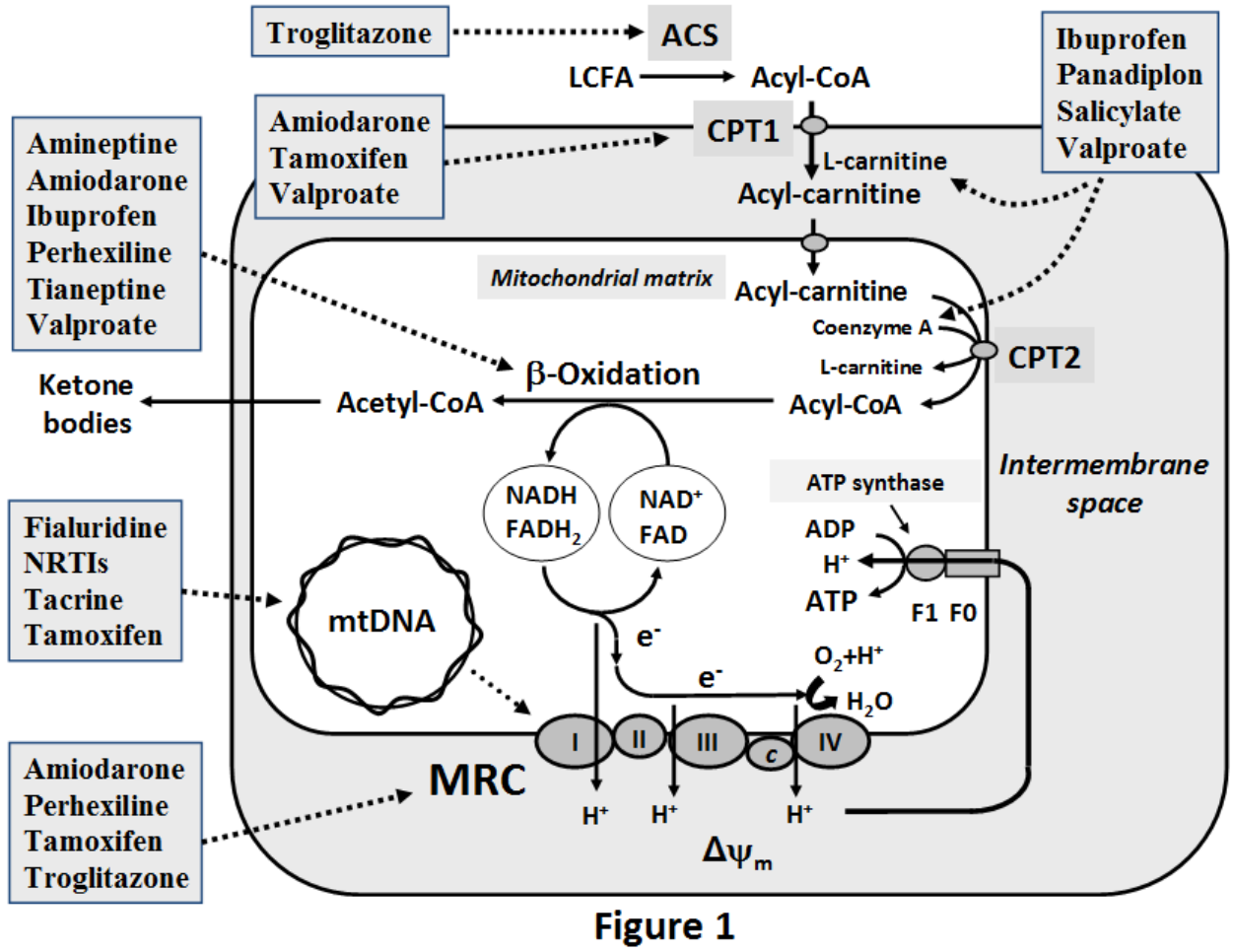

Figure 1

Legend to Figure 1

Mitochondrial β-Oxidation and main mechanisms whereby drugs can impair this metabolic pathway. *Adapted from* Begriche et al. [5]. Whereas short and medium-chain fatty acids (SCFAs and MCFAs) freely enter mitochondria (not shown), the entry of long-chain fatty acids (LCFAs) within these organelles requires a specific shuttle system involving four steps. (1) LCFAs are activated into LCFA- CoA thioesters by long-chain acyl-CoA synthetases (ACS). (2) The LCFA-CoA is converted into an acyl-carnitine by carnitine palmitoyltransferase-1 (CPT1) located in the outer mitochondrial membrane. (3) The acyl-carnitine is transferred across the inner mitochondrial membrane into the mitochondrial matrix by carnitine-acylcarnitine translocase. (4) Finally, carnitine palmitoyltransferase-2 (CPT2), located on the inner side of the inner mitochondrial membrane, transfers the acyl moiety from carnitine back to coenzyme A (CoA). Acyl-CoA thioesters are then oxidized into acetyl-CoA moieties *via* the β-oxidation process, irrespective of their chain length. Acetyl-CoA moieties can then generate ketone bodies (mainly acetoacetate and β-hydroxybutyrate), which are liberated into the plasma and used by extra-hepatic tissues for energy production. mtFAO generates NADH and $FADH_2$, which transfer their electrons (e^-) to the mitochondrial respiratory chain (MRC), thus regenerating

NAD^+ and FAD used for other β-oxidation cycles. Within the MRC, electrons are sequentially transferred to different polypeptide complexes (numbered from I to IV) embedded within the inner membrane. The final transfer of the electrons to oxygen takes place at the level of complex IV (cytochrome *c* oxidase). Importantly, the flow of electrons within the MRC is coupled to the extrusion of protons (H^+) from the matrix to the intermembrane space, which creates the mitochondrial transmembrane potential, $\Delta\psi_m$. When energy is needed, these protons re-enter the matrix through ATP synthase (complex V), thus liberating energy that is used to phosphorylate ADP into ATP. The mitochondrial DNA (mtDNA) encodes 13 polypeptides, which are inserted within complexes I, III, IV and V. Drugs can impair mtFAO through different mechanisms such as: 1) direct inhibition of β-oxidation enzyme(s), including ACS, CPT1 and different acyl-CoA dehydrogenases; 2) sequestration of the mtFAO cofactors L-carnitine and CoA; 3) inhibition of MRC activity, either directly or indirectly by way of mtDNA depletion; 4) impairment of PPARα expression and activity (not shown).